REMARKS

Reconsideration of the present application in view of the present amendment and the following remarks is respectfully requested. Claims 18-40 are pending, and claims 38-39 have been withdrawn by the Examiner. Applicants hereby cancel claims 18-20, 23, and 40 without prejudice to the filing of any divisional, continuation, or continuation-in-part application. Therefore, claims 21-22 and 24-37 are currently under examination, and new claims 41-59 are submitted herewith by amendment. Support for claims 41-59 may be found in the specification, for example, at page 4, lines 24-34; at page 5, line 15 through page 6, line 6; at page 6, line 25 through page 7, line 17; at page 9, line 31 through page 10, line 25; at page 11, lines 7-10; at page 15, line 11 through page 16, line 30; at page 17, line 18 through page 18, line 22; at page 21, lines 9-19; at page 25, lines 13-29; at page 26, line 1 through page 27, line 33; in the Examples (pages 31-65) and elsewhere. No new subject matter has been added.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned **"Version with markings to shows changes made."** Also, for the Examiner's convenience an Appendix is attached following the "Version with Markings" and containing all claims that are presently under examination in the present application further to entry of the present amendment.

A Final Office Action was mailed on May 29, 2001, in the original application and along with the Notice of Appeal, an Amendment and Response were filed on November 29, 2001. As noted in the Advisory Action mailed on December 12, 2001, the Amendment of November 29, 2001, was not entered into the record and did not place the application in condition for allowance. Accordingly, a Request for Continued Prosecution Application is submitted herewith in compliance with 37 CFR §1.53(d), and applicants believe the claims are now allowable as amended herein and in view of the following remarks.

PRIORITY

Applicants thank the Examiner for clarifying in the Final Office Action dated May 29, 2001, that a translation of the priority document DE 197 36 691.0 is not requested or required

at this time, and acknowledge the assertion in the Advisory Action dated December 12, 2001, that the DE document may not be relied on for priority in view of the 102(e) issues where a translation has not been provided.

Reference to the priority date of the DE document, however, has not been completely clarified. Applicants respectfully note that the Final Office Action misstates that the filing date of both PCT/EP98/05360 and DE 197 36 691.0 was August 24, 1998. PCT/EP98/05360 has an international filing date of August 24, 1998, but the filing date of the priority document, DE 197 36 691.0, was August 22, 1997. Applicants gratefully acknowledge the Examiner's note in the Advisory Action dated December 12, 2001, that the priority date of the DE document is August 25, 1997, rather than 1998 as set forth in the Final Office Action, but respectfully submit that the priority date is August 22, 1997, and not August 25, 1997.

RESTRICTION REQUIREMENT

In the Final Office Action, the Examiner withdrew newly submitted claims 38-39, asserting that these claims are directed to an independent or distinct invention from the invention originally claimed, and are therefore directed to a non-elected invention. Specifically, the Action alleges that the methods of claims 38 and 39 are directed to different methods with different objectives, method steps, and reagents. In the Advisory Action, the Examiner asserts further that the withdrawn claims require anticancer therapy treatment.

Applicants request that the withdrawal of claims 38 and 39 be without prejudice to the filing of any divisional, continuation, or continuation-in-part application.

Applicants also respectfully traverse the requirement for restriction of claims 38 and 39, and request that the Examiner join examination of these claims with the examination of the currently pending claims. Applicants submit that no undue burden would be placed on the Examiner to join examination of claims 38 and 39 with the pending claims because the subject matter of each of claims 38 and 39 is sufficiently related to the pending claims.

The present invention is directed in pertinent part to a method for determining an increased risk for or presence of a disseminated cancer cell or a micrometastasizing cancer cell in a body fluid from a subject, comprising (a) dividing a plurality of cells into at least a first fraction and a second fraction, wherein each of the fractions comprises at least one cell and wherein the

plurality of cells is from a body fluid of a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell, and wherein the second fraction comprises at least one cell that has been removed from said body fluid according to a method for isolating cancer cells from non-cancer cells; (b) detecting in the first fraction an absence or presence of at least one first nucleic acid selected from the group consisting of a first cancer-specific nucleic acid and a first cancer-associated nucleic acid; and (c) detecting, in the second fraction, an absence or presence of at least one second nucleic acid selected from the group consisting of a second cancer-specific nucleic acid and a second cancer-associated nucleic acid, wherein said first and second cancer-specific nucleic acids are different, wherein said first and second cancer-associated nucleic acids are different, wherein the presence of said first nucleic acid in the first fraction and an increased or decreased presence of the second nucleic acid in said second fraction relative to the presence or absence of said second nucleic acid in a non-cancer cell from the subject indicate an increased risk for having a disseminated cancer cell or a micrometastasized cancer cell.

Applicants submit that claim 38 relates to the method of the currently pending claims for determining whether a disseminated cancer cell or a micrometastasized cancer cell is present in the body fluid of a subject, wherein the method is performed before and after administering a candidate anticancer therapy to determine whether after administering the anticancer therapy, the presence of a cancer-specific or a cancer-associated nucleic acid has decreased relative to the presence or absence of the cancer-specific or cancer-associated nucleic acid in a non-cancer cell. Applicants respectfully submit that concurrent examination of claim 38 and the currently pending claims would not create a serious burden on the Examiner. Applicants submit that any searches to distinguish the present invention from other diagnostic methods of the prior art would invariably employ the same search terms with respect to methods for determining the risk for or presence of a disseminated or micrometastasized cancer cell as would be employed with respect to methods for determining the risk for or presence of a disseminated or micrometastasized cancer cell performed before and after a subject receives anticancer therapy. Any relevant references, should they exist, that might be identified in a search of a method for determining the risk for or presence of a disseminated or micrometastasized cancer cell would therefore be expected to have relevance also to the method for determining the risk for

or presence of a disseminated or micrometastasized cancer cell performed before and after a subject receives anticancer therapy.

Applicants also submit that claim 39 relates to the method of the currently pending claims, wherein before and after an anticancer agent contacts cells known to include or suspected of including a disseminated cancer cell or a micrometastatized cancer cell, the method of the currently pending claims is performed to identify an anticancer agent by detecting the absence or presence of a cancer-specific nucleic acid or a cancer-associated nucleic acid relative to the presence or absence of the cancer-specific nucleic acid or cancer-associated nucleic acid in a non-cancer cell. Applicants submit that concurrent examination of claim 39 and the currently pending claims would not create a serious burden on the Examiner. Prior art searches during examination that relate to methods performed before and after contacting a plurality of cells known to include or suspected of including a disseminated cancer cell or a micrometastasized cancer cell with an anticancer agent would invariably employ the same search terms that would be employed to distinguish the presently claimed invention from other diagnostic methods of the prior art. Any relevant references, should they exist, that might be identified in a search of a method for determining the risk for or presence of a disseminated or micrometastasized cancer cell by detecting the absence or presence of a cancer-specific or cancer-associated nucleic acid would therefore also be expected to have relevance to the method for identifying an anti-cancer agent by detecting the absence or presence of a cancer-specific nucleic acid or a cancer-associated nucleic acid relative to the presence or absence of the cancer-specific nucleic acid or cancer-associated nucleic acid in a non-cancer cell after contacting the anticancer agent.

Accordingly, applicants traverse the restriction of claims 38 and 39 into distinct inventions and submit that concurrent examination of claims 38 and 39, and of the currently pending claims, would not create a serious burden to the Examiner. Applicants therefore respectfully request that examination of claims 38 and 39 be joined with the examination of claims 21-22, 24-37, and 41-59. Reconsideration of the restriction requirement in view of the above remarks is respectfully requested.

REJECTIONS UNDER 35 U.S.C. § 112, SECOND PARAGRAPH

In the Final Office Action the Examiner rejected claims 18-37 and 40 under 35 U.S.C. § 112, second paragraph, for indefiniteness. More specifically, the Examiner is unclear with regard to the meaning of "detecting ... an absence or presence of at least one nucleic acid" and alleges that the claims do not define "whether the nucleic acid may be indirectly detected with an antigen that is specific for a nucleic acid," or whether a nucleic acid is specifically detected using a nucleic acid probe. The Action further asserts that these claims are unclear regarding when and how the act of removing a cancer cell from the sample occurs, absent a discrete step reciting such removal. Further with regard to these claims, the Examiner appears not to understand the relationship between particular nucleic acids and particular cells in the recitation "said nucleic acids in said cancer cell relative to the presence or absence of said nucleic acids in said non-cancer cell indicates an increased risk for having a disseminated cancer cell or a micrometastasized cancer cell". On this point, the Examiner asserts that the claimed method as a whole is confusing because the detection of a cancer-associated (or cancer-specific) nucleic acid when compared with normal cells would necessarily indicate an increased risk for dissemination or micrometastasis, because normal cells do not disseminate or micrometastasize.

The Examiner also rejected claims 20-37 for indefiniteness, asserting that the claims appeared to have two final process steps that indicate a risk for having a disseminated cancer cell or a micrometastasized cancer cell. The Examiner is unclear as to how the third determining step relates to the other steps, and further asserts that the claims are unclear with regard to the temporal relationship among the recited steps. The Action alleges that claims 23-31 lack proper antecedent basis where it is unclear to which nucleic acid of claims 18-20 the recitation "the nucleic acid" in claim 23 refers. The Examiner also rejected Claim 40 for indefiniteness, asserting that it is unclear how a particular type of malignant disease may be determined where the claim recites detecting any (*i.e.*, not a particular) cancer-specific nucleic acid.

Applicants respectfully traverse these grounds for rejection and submit that the present application as amended satisfies all requirements of 35 U.S.C. § 112, second paragraph. Applicants' invention is directed in pertinent part to a method for determining an increased risk for or presence of a disseminated cancer cell or a micrometastasizing cancer cell in a body fluid

from a subject, comprising (a) dividing a plurality of cells into at least a first fraction and a second fraction, wherein each of the fractions comprises at least one cell and wherein the plurality of cells is from a body fluid of a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell, and wherein the second fraction comprises at least one cell that has been removed from said body fluid according to a method for isolating cancer cells from non-cancer cells; (b) detecting in the first fraction an absence or presence of at least one first nucleic acid selected from the group consisting of a first cancer-specific nucleic acid and a first cancer-associated nucleic acid; and (c) detecting, in the second fraction, an absence or presence of at least one second nucleic acid selected from the group consisting of a second cancer-specific nucleic acid and a second cancer-associated nucleic acid, wherein said first and second cancer-specific nucleic acids are different, wherein said first and second cancer-associated nucleic acids are different, wherein the presence of said first nucleic acid in the first fraction and an increased or decreased presence of the second nucleic acid in said second fraction relative to the presence or absence of said second nucleic acid in a non-cancer cell from the subject indicate an increased risk for having a disseminated cancer cell or a micrometastasized cancer cell.

Applicants submit that in view of the present amendment, the instant application meets the requirements for clarity and precision under 35 U.S.C. § 112, second paragraph. Applicants further submit that one of ordinary skill in the art would understand on the basis of the accepted meaning in the art and disclosures of the instant application that "detecting ... an absence or presence of at least one first nucleic acid" and similar claim language indicates that the method comprises direct detection of nucleic acids (see, *e.g.*, specification, page 15, lines 11-13). In contrast, an indirect method, would include, for example, immunological detection of a protein expressed by a gene (see, *e.g.*, specification, page 14, line 25 through page 15, line 9.) The methods on page 18 of the specification to which the Examiner specifically refers in the Action do not describe either indirect or direct detection of nucleic acids, but instead support the preceding step in the claimed method, namely that of dividing the cells of a body fluid into fractions, wherein cancer cells are removed from body fluids, and wherein cells are quantified to ensure that each recited fraction comprises at least one cell. Applicants therefore respectfully submit that as amended herewith, the claims of the present application particularly point out and

distinctly claim a method comprising, in pertinent part, detection of a nucleic acid in each fraction, as disclosed in the specification and recited in the claims.

Contrary to the assertion in the Action, applicants also respectfully submit that the amended claims submitted herewith distinctly point out when and how the act of removing a cancer cell from the sample occurs. Applicants further submit that the instant claims as presently amended provide proper antecedent basis for all claim elements, and thereby eliminate confusion with respect to the final process step. In pertinent part, for instance, the claims recite (i) that the plurality of cells removed from the body fluid of a subject is divided into at least a first and second fraction, each comprising at least one cell, wherein the second fraction comprises at least one cell that has been removed from the body fluid according to a method for isolating cancer cells from non-cancer cells; (ii) detecting in the first fraction an absence or presence of at least one first nucleic acid; and (iii) detecting in the second fraction an absence of presence of a second, different nucleic acid. The final process step points out (a) that the presence of the first nucleic acid in the first fraction, and the increased or decreased presence of the second nucleic acid in the second fraction relative to the presence or absence of the second nucleic acid in a non-cancer cell, indicate an increased risk for having a disseminated or a micrometastatized cancer cell. Support for such amendments to the claims may be found in the specification, for example, at page 17, line 18 through page 18, line 35. Applicants respectfully submit that the metes and bounds of the claimed invention are clear, and that the present amendment therefore obviates the grounds for the instant rejection under 35 U.S.C. §112, second paragraph.

The Examiner rejected claims 20-37 under 35 U.S.C. §112, second paragraph, asserting that they were indefinite because claim 20 appeared to have two final process steps, alleging further that the temporal relationship among the steps was unclear. Applicants respectfully traverse this ground of rejection and submit that the new and amended claims submitted herewith each recite a single final step that clearly relates to other steps in the claimed method.

For example, new claim 43 recites, in pertinent part, a method for determining an increased risk for or presence of a disseminated cancer cell or a micrometastasizing cancer cell in a body fluid from a subject comprising: (a) dividing a plurality of cells into at least a first fraction and a second fraction, each comprising at least one cell, wherein the second fraction comprises at

least one cell that has been removed from the body fluid according to a method for isolating cancer cells from non-cancer cells; (b) detecting in the first fraction an absence or presence of at least one first cancer-specific nucleic acid; (c) detecting in the second fraction an absence or presence of at least one second cancer-specific nucleic acid; and (d) detecting an absence or presence of at least one cancer-associated nucleic acid in at least one sample selected from the group consisting of (i) the first fraction and (ii) the second fraction.

The final step further clearly points out how to compare the presence or absence of each nucleic acid detected to determine whether the subject has an increased risk for having a disseminated cancer cell or a micrometastasized cancer cell, as recited: wherein the presence of the first cancer-specific nucleic acid and of the cancer-associated nucleic acid in the first fraction and an increased or decreased presence of the second cancer-specific nucleic acid and of the second cancer-associated nucleic acid in the second fraction relative to the presence or absence of the second cancer-specific nucleic acid and of the second cancer-associated nucleic acid in a non-cancer cell from the subject indicate an increased risk for having a disseminated cancer cell or a micrometastasized cancer cell. Applicants therefore respectfully submit that this rejection of claims 20-37 and 40 is rendered moot by the present amendment, and request that this rejection be withdrawn.

The Examiner rejected claims 23-30 under 35 U.S.C. §112, second paragraph, alleging that claim 23 lacks proper antecedent basis because the claim is directed to "the nucleic acid"; however, claims 18-20, upon which 23 depends, cite numerous nucleic acids. Applicants respectfully submit that rejection of claims 23-31 is obviated by the present amendment canceling claim 23. Also, by the amendment submitted herewith, new claims 52-54, 57 and 60 have been added that particularly point out and distinctly claim the subject matter which applicants regard as the invention. Support for these new claims may be found in the specification, for example, at page 6, lines 27-35.

For similar reasons, rejection of claim 40 under 35 U.S.C. §112, second paragraph, for alleged indefiniteness is also obviated by the present amendment canceling claim 40. The Final Office Action asserted that claim 40 does not clearly recite how to determine the type of malignant disease when using any cancer-specific nucleic acid. New claim 44 is directed to a method comprising typing a malignant disease in a subject known to have, or suspected of

being at risk for having, a malignant disease. The claim clearly points out and distinctly recites, in pertinent part, that at least one of the first cancer-specific and cancer-associated nucleic acids detected in the first fraction comprises an organotypical gene, and that the presence of at least one of the first nucleic acids comprising an organotypical gene indicates the type of malignant disease from which the cancer cell is derived. Support for claim 44 may be found in the specification, for example, at page 8, line 30 through page 9, line 26 and at page 10, line 9 through page 11, line 25.

In view of the present amendment and the remarks provided herein, Applicants respectfully submit that the new and amended claims submitted herewith bring the present application into compliance with the requirements for clarity and precision under 35 U.S.C. § 112, second paragraph. Applicants therefore request that these rejections be withdrawn.

REJECTIONS UNDER 35 U.S.C. § 102(e)

Claims 18-26, 32, 37, and 40 stand rejected for lack of novelty under 35 U.S.C. § 102(e) as allegedly anticipated by Rimm et al. (U.S. Patent No. 6,197,523). In particular, the Final Office Action asserts that the instant claims read on Rimm et al. where the cited reference teaches a method for detection, identification, enumeration, and confirmation of circulating cancer or hematologic progenitor cells in whole blood by detecting a cancer-specific epitope or by "other methods such as PCR".

Applicants respectfully traverse this ground for rejection. The present invention is directed to a method for determining an increased risk for or presence of a disseminated cancer cell or a micrometastasizing cancer cell in a body fluid from a subject, comprising (a) dividing a plurality of cells into at least a first fraction and a second fraction, wherein each of the fractions comprises at least one cell and wherein the plurality of cells is from a body fluid of a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell, and wherein the second fraction comprises at least one cell that has been removed from said body fluid according to a method for isolating cancer cells from non-cancer cells; (b) detecting in the first fraction an absence or presence of at least one first nucleic acid selected from the group consisting of a first cancer-specific nucleic acid and a first cancer-associated nucleic acid; and (c) detecting, in the second fraction, an absence or presence

of at least one second nucleic acid selected from the group consisting of a second cancer-specific nucleic acid and a second cancer-associated nucleic acid, wherein said first and second cancer-specific nucleic acids are different, wherein said first and second cancer-associated nucleic acids are different, wherein the presence of said first nucleic acid in the first fraction and an increased or decreased presence of the second nucleic acid in said second fraction relative to the presence or absence of said second nucleic acid in a non-cancer cell from the subject indicate an increased risk for having a disseminated cancer cell or a micrometastasized cancer cell. The present invention is also directed to related methods.

Rimm et al. fail to teach each and every element of the instant claims, and Rimm et al. therefore fail to anticipate the present invention. Rimm et al. merely describe a method for visually or photometrically detecting circulating cancer and/or hematologic progenitor cells in a whole blood sample. One step involves the detection of characteristic epitopic highlighting on the cells to determine the epithelial origin using one or more epitope-specific labeling agents (Rimm et al., column 4, lines 45-55). The other step involves morphological examination of cells either to identify suspicious cells, or to confirm the malignant nature of the cells. According to the teachings of Rimm et al. it is generally desirable to use both the epitopic and morphometric analyses in assaying the blood sample (column 5, lines 3-21).

Furthermore, Rimm et al. concede that although morphometric analysis may be sufficient for identification of cancer cells, other methods of verification may also be necessary. To this end, the cells may be removed from the sampling tube for additional analysis by other methods such as the PCR method known to the prior art, or by biochemical assay (column 12, lines 1-11). Rimm et al., however, fail to provide any further disclosure pertaining to such additional analysis techniques. Accordingly, Applicants submit that Rimm et al. fail to teach or suggest a method for determining an increased risk for of presence of disseminated or micrometastasized cancer cells by detecting at least one cancer-specific or cancer-associated nucleic acid in a body fluid sample *and* by detecting at least one second, different cancer-specific or cancer-associated nucleic acid in a second fraction of the sample comprising cells removed from the body fluid according to a method that isolates cancer cells from non-cancer cells. Therefore, Applicants respectfully request that this rejection be withdrawn.

The Examiner also rejected claims 18-37, and 40 under 35 U.S.C. § 102(e) as being anticipated by Schmitz et al. (U.S. Patent No. 6,190,870). In particular, the Action asserts that separation of tumor cells from peripheral blood by magnetic sorting using magnetically labeled antibodies directed to tumor antigens, as disclosed by Schmitz et al., meets every limitation of the instant claims.

Applicants respectfully traverse this ground for rejection and submit that Schmitz et al. do not teach or suggest all limitations of the presently claimed invention, *i.e.*, the claims submitted herewith by amendment are clearly distinguishable over Schmitz et al. As also noted above, and as disclosed in the specification and recited in the instant claims, the present invention is directed in pertinent part to a method for determining an increased risk for or presence of a disseminated cancer cell or a micrometastasizing cancer cell in a body fluid from a subject, comprising the recited steps of (a) dividing a plurality of cells into a first fraction and a second fraction, which second fraction comprises at least one cell that has been removed from the body fluid according to a method for isolating cancer cells from non-cancer cells; (b) detecting in the first fraction absence or presence of at least one first nucleic acid, which may be a first cancer-specific or a first cancer-associated nucleic acid; and (c) detecting in the second fraction absence or presence of at least one second nucleic acid that is a second cancer-specific nucleic acid or a second cancer-associated nucleic acid, wherein the first and second cancer-associated nucleic acids are different, and wherein the presence of said first nucleic acid in the first fraction and an increased or decreased presence of the second nucleic acid in said second fraction relative to the presence or absence of said second nucleic acid in a non-cancer cell from the subject indicate an increased risk for having a disseminated cancer cell or a micrometastasized cancer cell. As discussed herein and described in the instant application, the present invention is also directed to related embodiments.

Applicants submit that it is well settled that a reference anticipates a claimed invention only when each and every claim limitation is present in the single reference. Because Schmitz et al. fail to provide every limitation of the instant claims, applicants submit that Schmitz et al. fail to disclose the present invention. In particular, Schmitz et al. fail to teach a method for determining an increased risk for or presence of a disseminated or micrometastasized cancer cell that comprises detecting at least one cancer-specific or cancer-associated nucleic acid

in a sample *and* that further comprises detecting at least one second, different cancer-specific or cancer-associated nucleic acid in a fraction of the sample comprising cells isolated according to a method that removes cancer cells. Schmitz et al. merely disclose a method for enrichment of tumor cells from a sample whereby only the enriched fraction is characterized. Thus, Schmitz et al. fail to teach or suggest detecting at least one cancer-specific or cancer-associated nucleic acid in a sample before cancer cells are removed from the sample *and* detecting a second, different cancer-specific or cancer-associated nucleic acid in a sample after enrichment. Accordingly, Applicants submit that the present invention is novel where the cited reference fails to provide each claim limitation, and therefore respectfully request that this rejection be withdrawn.

REJECTIONS UNDER 35 U.S.C. § 103

The Examiner rejected claims 18-32, 37, and 40 under 35 U.S.C. § 103(a) for obviousness over Zhuang et al. (*ACTA Cytologica*, 38:671-75 (1994)) in view of Popescu et al. (*Cancer Genet. Cytogenet.* 93:10-21 (1997)) or Torczynski et al. (U.S. Patent No. 5,589 579). More specifically, basing this rejection on the interpretation that the claims require nucleic acid detection, the Examiner alleges that a person having ordinary skill in the art would have been motivated to combine the teachings of Zhuang et al. regarding detecting tumor cells and detecting a cancer-specific or cancer-associated nucleic acid therein, with the teachings of either Popescu et al. or Torczynski et al. regarding identifying tumor cells using a cancer-specific nucleic acid, to arrive at the claimed invention.

Applicants respectfully traverse these grounds for rejection and submit that the references cited by the Action, alone or in combination, fail to teach or suggest the subject matter of the presently claimed invention according to the amendment submitted herewith. As is also noted above, the present invention is directed in pertinent part to a method for determining an increased risk for or presence of a disseminated cancer cell or a micrometastasizing cancer cell in a body fluid from a subject, comprising the recited steps of dividing a plurality of cells into at least a first and a second fraction; detecting in the first fraction the absence or presence of a first nucleic acid; and detecting in the second fraction an absence or presence of a second nucleic acid, wherein the presence of the first nucleic acid in the first fraction and increased or decreased

presence of the second nucleic acid in the second fraction relative to that in a non-cancer cell indicate increased risk.

Applicants submit that a person having ordinary skill in the art would not have been motivated by the prior art, including the references cited in the Action, to arrive at the present invention with any reasonable expectation of success. Accordingly, and for reasons discussed herein, applicants respectfully submit that the Examiner has not established a *prima facie* case of obviousness. (*See In re Mayne*, 104 F.3d 133, 1341-43, 41 U.S.P.Q.2d 1451 (Fed. Cir. 1997) (PTO has the burden of showing a *prima facie* case of obviousness.)). The Examiner must show (1) that the combined references teach or suggest all claim limitations; (2) that the references provide some teaching, suggestion, or motivation to combine or modify the teachings of the prior art to produce the claimed invention; and (3) that the combined teachings of the references indicate that by combining the references, a person having ordinary skill in the art will achieve the claimed invention with a reasonable expectation of success. When rejection of claims depends upon a combination of prior art references, a teaching, motivation, or suggestion to combine the references must exist. (*See In re Rouffet*, 149 F.3d 1350, 1355, 47 U.S.P.Q.2d 1453 (Fed. Cir. 1998).

Zhuang et al., Popescu et al., and Torczynski et al., alone or in combination, fail to teach or suggest a method for determining an increased risk for or presence of a disseminated or micrometastasized cancer cell by detecting at least one cancer-specific or cancer-associated nucleic acid in a body fluid sample and by detecting at least one second, different cancer-specific or cancer-associated nucleic acid in a fraction of the sample comprising cells isolated according to a method that isolates cancer cells from non-cancer cells. As the Action concedes, Zhuang et al. merely teach identifying cancer cells on the basis of morphology, but do not specifically teach identifying tumor cells using a first cancer-specific nucleic acid. Zhuang et al. also fail to teach or suggest a method comprising dividing a plurality of cells from a body fluid of a subject known to have or suspected of being at risk for having a *disseminated* cancer cell or a *micrometastasized* cancer cell, where Zhuang et al. merely describe microdissection of material from a *primary* tumor that has been enzymatically dissociated and that cannot reflect disseminated cancer cells.

Moreover, applicants submit that Zhuang et al. not only fail to teach use of a body fluid from which a fraction comprising a cancer cell can be removed (*i.e.*, that contains both

cancer and non-cancer cells), but that Zhuang et al. teach away from the use of such a sample by demonstrating the unsuitability of such a sample for the analysis described therein. Specifically, Zhuang et al. teach that in a heterogenous cytological tumor sample comprising both cancer and non-cancer cells, the sensitivity of nucleic acid detection (*e.g.*, PCR) undesirably compromises the analysis of the sample (see Zhuang et al., page 673, discussion, column 2), *i.e.*, a sample that is non-homogeneous with respect to cell type presents drawbacks using the method of Zhaung et al. Thus, Zhuang et al. provide no motivation to a person having ordinary skill in the art to detect, according to the subject invention method, a cancer-specific nucleic acid in a body fluid that comprises a disseminated or micrometastatized cancer cell and a non-cancer cell, because the analysis would if anything be hindered by the heterogeneity of the sample. Hence, the brunt of the teachings of Zhuang et al. suggest that a heterogeneous cell sample is highly undesirable for the DNA extraction/ PCR method described in that reference, in marked contrast to the essence of the present invention, which is directed to characterization of disseminated or micrometastasizing cancer cells that occur in samples that are non-homogeneous with respect to cell type, such as the recited body fluid.

Accordingly, applicants respectfully submit that modification of the method of Zhuang et al., as the Action asserts would have been obvious to an ordinarily skilled artisan to arrive at applicants' invention, would, on the contrary, render the method of Zhuang et al. unsatisfactory for its intended purpose, which is analysis of DNA from a pure population of tumor cells. When a proposed modification would render the prior art disclosure unsatisfactory for its intended purpose, no motivation or suggestion to make the proposed modification can exist. (*See In re Gordon*, 733 F.2d 900, 902, 221 U.S.P.Q. 1125 (Fed. Cir. 1984).

Applicants further submit that the Action fails to establish a *prima facie* case of obviousness because Zhuang et al. in view of Popescu et al. or Torczynski et al. do not teach or suggest all claim elements. As discussed above, Zhuang et al. fail to teach using a first cancer-specific nucleic acid, and Zhuang et al. teach away from using a sample comprising cancer and non-cancer cells. Neither Popescu et al. nor Torczynski et al. remedy these deficiencies of Zhuang et al., because Popescu et al. or Torczynski et al. alone or in combination fail to teach or suggest a method for determining a risk for or presence of a disseminated or micrometastasized cancer cell by detecting at least one cancer-specific or cancer-associated nucleic acid in a body

fluid sample *and* by detecting at least one second, different cancer-specific or cancer-associated nucleic acid in a fraction of the sample comprising cells isolated according to a method that removes cancer cells. Popescu et al. simply provide a general review, without more, of the use of PCR and FISH methodologies to detect chromosomal alterations in a cancer cell, thus necessitating the absence of non-cancer cells, but nowhere do Popescu et al. suggest any desirability of employing PCR or FISH in cancer-enriched and unfractionated cells, or of analyzing disseminated or micrometastasized cells. Torczynski et al. merely disclose several molecular markers that may be used for diagnosis of lung cancer, including several well-known in the art, such as CEA, NCA, and the ras and myc families of oncogenes; these authors also disclose a cell surface protein (HCAVIII) specific for non-small cell lung cancer. However, Torczynski et al. also fail in any way to suggest the desirability of combining detection of these markers with any methodology known to the art, to arrive at a method comprising the limitations of the present invention for characterization of cancer cells.

Applicants therefore submit that any combination of Zhuang et al., Popescu et al., and Torczynski et al. simply does not teach or suggest the present invention, nor would such combination have motivated a person having ordinary skill in the art to modify Zhuang et al. to arrive at the instant invention with a reasonable expectation of success. Applicants respectfully submit that the present claims are nonobvious under 35 U.S.C. §103 and request that this rejection be withdrawn.

The Examiner also rejected claims 18-26, 32, 37, and 40 under 35 U.S.C. § 103(a) for obviousness over Ts'o et al. (U.S. Patent No. 5,962,237) in view of Rimm et al. (U.S. Patent No. 6,197,523). More specifically, basing this rejection on the interpretation that the claims do not require detection of nucleic acids using nucleic acids, the Examiner alleges that a person having ordinary skill in the art would have been motivated to combine the teachings of Ts'o et al. regarding negatively selecting tumor cells and detecting a cancer-specific or cancer-associated nucleic acid therein, with the teachings of Rimm et al. regarding identifying a cancer-specific antigenic epitope, to arrive at the claimed invention.

Applicants respectfully traverse this ground for rejection and submit that Ts'o et al. and Rimm et al., alone or in combination, do not teach or suggest the present invention. The Action concedes that Ts'o et al. do not specifically teach detecting a cancer-specific nucleic acid

or a cancer-associated nucleic acid prior to the enriching step. For reasons given above, applicants respectfully submit that Rimm et al. cannot remedy this deficiency of Ts'o et al., because Rimm et al. merely teach morphological detection or epitope-specific labeling of hematopoietic progenitor cells in whole blood, but Rimm et al. fail to teach detecting first and second nucleic acids according to the methods of the present invention. Even assuming, *arguendo*, that Rimm et al. might *suggest* some type of nucleic acid analysis where Rimm et al. merely allude, without providing any details, to verification of samples using PCR (Col. 12, lines 1-11), Applicants submit that given the myriad possible configurations of sample selection and of PCR proband selection, Rimm et al. alone or in combination with Ts'o et al., or any other prior art references, fall far short of providing the requisite motivation for a person having ordinary skill in the art to arrive at the presently claimed invention with a reasonable expectation of success.

Specifically, the prior art fails to suggest detecting a first cancer-specific or a first cancer-associated nucleic acid in a body fluid containing cells according to the instant claims *and* detecting a second cancer-specific or cancer-associated nucleic acid in a sample fraction comprising cells that have been removed from the body fluid according to a method for isolataing cancer cells from non-cancer cells. Applicants submit further that only through the use of impermissible hindsight in view of the instant application can an allegation be made that the subject invention method might have been obvious in view of the art at the time of filing. On this point, Applicants submit that the Action fails to point specifically to any prior art suggestions to select, *e.g.*, the recited combinations of sample fractions, first and second nucleic acids and method for isolating cancer cells from non-cancer cells, *per se*, to arrive at the subject matter of the instant claims. Therefore, Applicants respectfully submit that application of Ts'o et al. in view of Rimm et al. fail to anticipate the claimed invention, and request that this rejection be withdrawn.

The Action also rejected claims 33-36 under 35 U.S.C. § 103(a) as being obvious over Zhuang et al. in view of Popescu et al. or Torczynski et al. (as applied to claims 18-32, 37, and 40) and further in view of Schmitz et al. (U.S. Patent No. 6,190,870). The Examiner asserts that modification of the teachings of Zhuang, Popescu, and Torczynski by those of Schmitz

would have been *prima facie* obvious to one of ordinary skill in the art at the time of filing the present application.

Applicants respectfully traverse this ground for rejection and submit that any of the cited references, alone or in combination, fail to teach or suggest applicants' invention. In particular, cell selection markers and cancer-related genes described by Schmitz et al. are merely cumulative examples, respectively, of methods for removing cancer cells and of cancer-specific or cancer-associated nucleic acids, which are described in the instant application. However, Schmitz et al. fail to remedy the deficiencies of the other cited references, as discussed above, such that the prior art fails to suggest the particular combination of elements that gives rise to the present invention, nor does the Action point to teachings in the art that would have made the claimed invention obvious.

Claims 33-36 are directed to a method for determining an increased risk for or presence of a disseminated or micrometastasized cancer cell comprising the recited steps of dividing a plurality of cells and detecting absence or presence of first and second nucleic acids, as discussed above, including detection of certain second nucleic acids that may be metastasis-associated genes. Applicants submit that a person having ordinary skill in the art at the time the invention was filed would not have been motivated to combine the above references to achieve applicants' invention with a reasonable expectation of success.

In particular, and as discussed in detail above, applicants submit that Zhuang et al. in view of Popescu et al. and/or Torczynski et al. fail to teach or suggest a method for determining an increased risk for or presence of a disseminated or micrometastasized cancer cell by detecting at least one cancer-specific or cancer-associated nucleic acid in a body fluid sample *and* by detecting at least one second, different cancer-specific or cancer-associated nucleic acid in a second fraction of the sample comprising cells isolated from the body fluid according to a method that isolates cancer cells from non-cancer cells.

Briefly, Zhuang et al. teach a primary tumor as the source of cancer cells, but do not suggest or teach using a body fluid and a derivative fraction thereof containing removed cancer cells, *i.e.*, Zhuang et al. fail to contemplate a diagnostic method using a sample that contains both cancer and non-cancer cells, and dividing such a sample into two fractions, and detecting first and second nucleic acids, as recited in the claims submitted herewith. Zhuang et

al. also do not teach identifying tumor cells using a first cancer-specific nucleic acid. Furthermore, Zhuang et al. state that the sensitivity of PCR in a heterogenous cytological tumor sample comprising both cancer and non-cancer cells is a drawback to their analysis. Therefore, modification of the method of Zhuang et al. to achieve applicants' invention would render Zhuang's method inoperative for its intended purpose. Furthermore, Zhuang et al. in combination with either Popescu et al. or Torczynski et al. do not teach or suggest all claim limitations of the present invention. As noted above, neither Popescu et al. nor Torczynski et al. teach or suggest a method for determining an increased risk for or presence of a disseminated or micrometastasized cancer cell by detecting at least one cancer-specific or cancer-associated nucleic acid in a body fluid sample *and* by detecting at least one second, different cancer-specific or cancer-associated nucleic acid in a fraction of the sample comprising cells isolated according to a method that isolates cancer cells from non-cancer cells.

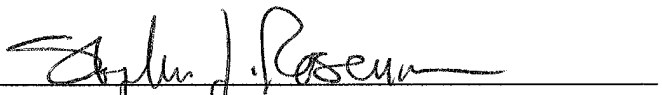
Applicants submit that Schmitz et al. also fail to teach or in an way suggest detecting at least one cancer-specific or cancer-associated nucleic acid in a sample from which cancer cells need not be removed from the sample *and* detecting a second, different cancer-specific or cancer-associated nucleic acid in a fraction derived from the sample and enriched for cancer cells. Thus, the Schmitz reference does not remedy the deficiencies of Zhuang et al., Popescu et al., and Torczynski et al., nor do Schmitz et al. render *prima facie* obvious the independent claims upon which claims 33-36 depend. The disclosure of Schmitz et al. is limited to the teaching, in cells selected on the basis of surface marker expression by art-accepted methods, that certain metastatic factors expressed in malignancy may be of interest. However, Schmitz et al. fail to suggest the desirability of including the detection of nucleic acids encoding these factors in a method for determining a risk for or presence of disseminated or micrometastasized cancer cell according to the method of applicants' invention, which is disclosed in the instant application and recited in the claims as amended herewith. Hence, none of the cited references alone or in combination teach or suggest each claim limitation of the present invention. Accordingly, Applicants respectfully submit that the claimed invention satisfies the requirements of 35 U.S.C. § 103 for nonobviousness and request that the rejection of claims 33-36 be withdrawn.

In view of the present amendment and the above remarks, applicants respectfully submit that all of the claims in the application are allowable. Favorable consideration and a Notice of Allowance are earnestly solicited.

Respectfully submitted,

Michael Giesing et al.

Seed Intellectual Property Law Group PLLC



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

Claims 18-20, 23, and 40 have been canceled.

Claims 21-22, 24, 27-30, 32 and 37 have been amended and new claims 41-59 added as follows:

21. (Amended) The method of claim ~~20~~43 wherein the first and second cancer-specific nucleic acids are the same.

22. (Amended) The method of claim ~~20~~43 wherein the first and second cancer-specific nucleic acids are different.

24. (Amended) The method of any one of claims ~~23~~54-56 wherein the RNA comprises mRNA.

27. (Amended) The method of any one of claims ~~23~~54-56 wherein the DNA that is detected comprises genomic DNA selected from the group consisting of genomic DNA comprising a genomic mutation, genomic DNA comprising a gene that has undergone amplification, genomic DNA comprising a gene that has undergone loss of heterozygosity, genomic DNA comprising a translocated gene and genomic DNA comprising a gene polymorphism.

28. (Amended) The method of any one of claims ~~23~~54-56 wherein at least one nucleic acid that is detected comprises DNA, said DNA comprising genomic DNA selected from the group consisting of (i) the second cancer-specific nucleic acid and (ii) a cancer-associated nucleic acid that is present in at least one cancer cell ~~removed from the plurality of cells and that is absent from any non-cancer cells of the plurality of cells~~ in the second fraction.

29. (Amended) The method of any one of claims ~~23~~54-56 wherein the DNA is genomic DNA that comprises all or a portion of an oncogene.

30. (Amended) The method of any one of claims ~~23~~54-56 wherein the DNA is genomic DNA that comprises all or a portion of a tumor suppressor gene.

32. (Amended) The method of any one of claims ~~18-20~~41-43 wherein at least one nucleic acid selected from the group consisting of a ~~cancer-specific nucleic acid and a~~ (i) first cancer-associated nucleic acid and (ii) a second cancer-associated nucleic acid comprises a coding portion of a gene selected from the group consisting of a tissue-specific gene, a metastasis-associated gene, a steroid hormone receptor gene, a drug resistance gene, an immunomodulation gene, a cell proliferation gene and an apoptosis gene, or a complementary nucleic acid thereto.

37. (Amended) The method of any one of claims ~~18-20~~41-43 wherein the cancer cell is removed from the body fluid by a method selected from the group consisting of microfiltration, density gradient centrifugation and antigen-specific immunoadsorption.

41. (New) A method for determining an increased risk for or presence of a disseminated cancer cell or a micrometastasizing cancer cell in a body fluid from a subject, comprising:

(a) dividing a plurality of cells into at least a first fraction and a second fraction, wherein each of the fractions comprises at least one cell and wherein the plurality of cells is from a body fluid of a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell, and wherein the second fraction comprises at least one cell that has been removed from said body fluid according to a method for isolating cancer cells from non-cancer cells;

(b) detecting in the first fraction an absence or presence of at least one first nucleic acid selected from the group consisting of a first cancer-specific nucleic acid and a first cancer-associated nucleic acid; and

(c) detecting, in the second fraction, an absence or presence of at least one second nucleic acid selected from the group consisting of a second cancer-specific nucleic acid and a second cancer-associated nucleic acid, wherein said first and second cancer-specific nucleic acids are different, wherein said first and second cancer-associated nucleic acids are different, wherein the presence of said first nucleic acid in the first fraction and an increased or decreased presence of the second nucleic acid in said second fraction relative to the presence or absence of said second nucleic acid in a non-cancer cell from the subject indicate an increased risk for having a disseminated cancer cell or a micrometastasized cancer cell.

42. (New) A method for determining an increased risk for or presence of a disseminated cancer cell or a micrometastasizing cancer cell in a body fluid from a subject, comprising:

(a) dividing a plurality of cells into at least a first fraction and a second fraction, wherein each of the fractions comprises at least one cell and wherein the plurality of cells is from a body fluid of a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell, and wherein the second fraction comprises at least one cell that has been removed from said body fluid according to a method for isolating cancer cells from non-cancer cells;

(b) detecting in the first fraction an absence or presence of at least one first cancer-specific nucleic acid; and

(c) detecting in the second fraction an absence or presence of at least one second cancer-specific nucleic acid, wherein said first and second cancer-specific nucleic acids are different, wherein the presence of said first cancer-specific nucleic acid in said first fraction and an increased or decreased presence of said second cancer-specific nucleic acid in said second fraction relative to the presence or absence of said second cancer-specific nucleic acid in a non-cancer cell from the subject indicate an increased risk for having a disseminated cancer cell or a micrometastasized cancer cell.

43. (New) A method for determining an increased risk for or presence of a disseminated cancer cell or a micrometastasizing cancer cell in a body fluid from a subject, comprising:

(a) dividing a plurality of cells into at least a first fraction and a second fraction, wherein each of the fractions comprises at least one cell and wherein the plurality of cells is from a body fluid of a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell, and wherein the second fraction comprises at least one cell that has been removed from said body fluid according to a method for isolating cancer cells from non-cancer cells;

(b) detecting in the first fraction an absence or presence of at least one first cancer-specific nucleic acid;

(c) detecting in the second fraction an absence or presence of at least one second cancer-specific nucleic acid; and

(d) detecting an absence or presence of at least one cancer-associated nucleic acid in at least one sample selected from the group consisting of (i) the first fraction and (ii) the second fraction, wherein the presence of said first cancer-specific nucleic acid and of said cancer-associated nucleic acid in said first fraction and an increased or decreased presence of said second cancer-specific nucleic acid and of said second cancer-associated nucleic acid in said second fraction relative to the presence or absence of said second cancer-specific nucleic acid and of said second cancer-associated nucleic acid in a non-cancer cell from the subject indicate an increased risk for having a disseminated cancer cell or a micrometastasized cancer cell.

44. (New) The method of any one of claims 41-43 wherein a nucleic acid selected from the group consisting of (i) a first cancer-specific nucleic acid and (ii) a first cancer-associated nucleic acid comprises an organotypical gene, and wherein the presence of at least one of said first nucleic acids comprising an organotypical gene indicates the type of malignant disease from which the cancer cell is derived.

45. (New) The method of any one of claims 41-43 wherein a nucleic acid selected from the group consisting of (i) a first cancer-associated nucleic acid and (ii) a second

cancer-associated nucleic acid comprises a metastasis-associated gene, and wherein the presence of said first cancer-associated nucleic acid comprising the metastasis-associated gene indicates an increased risk that a disseminated cancer cell has the ability to metastasize, and wherein an increased or decreased presence of said second cancer-associated nucleic acid comprising the metastasis-associated gene in said cancer cell relative to the presence or absence of said second cancer-associated nucleic acid comprising the metastasis-associated gene in a non-cancer cell from the subject indicates an increased risk that a disseminated cancer cell has the ability to metastasize.

46. (New) The method of claim 45 wherein the metastasis-associated gene encodes a gene product selected from the group consisting of an angiogenesis factor, a motility factor, a growth factor, a matrix degradation factor and an adhesion factor.

47. (New) The method of claim 46 wherein the matrix degradation factor is selected from the group consisting of a proteinase and a proteinase inhibitor.

48. (New) The method of claim 46 wherein the adhesion factor is an adherin.

49. (New) The method of claim 45 wherein the nucleic acid is selected from the group consisting of DNA and RNA.

50. (New) The method of claim 49 wherein the RNA comprises mRNA.

51. (New) The method of claim 50 wherein the mRNA encodes a gene product selected from the group consisting of bFGF, bFGF-R, VEGF, VEGF-R1, VEGF-R2, MMP2 and TIMP3.

52. (New) The method according to any one of claims 41-42 wherein steps (a) - (c) are performed before and after administering a candidate anticancer therapy to a subject

known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell.

53. (New) The method according to claim 43 wherein steps (a) - (d) are performed before and after administering a candidate anticancer therapy to a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell.

54. (New) The method of claim 41 wherein the first nucleic acid is RNA and wherein the second nucleic acid is selected from the group consisting of DNA and RNA.

55. (New) The method of claim 42 wherein the first cancer-specific nucleic acid is RNA and wherein the second cancer-specific nucleic acid is selected from the group consisting of DNA and RNA.

56. (New) The method of claim 43 wherein the first cancer-specific nucleic acid is selected from the group consisting of DNA and RNA, wherein the second cancer-specific nucleic acid is selected from the group consisting of DNA and RNA, and wherein the cancer-associated nucleic acid is selected from the group consisting of DNA and RNA.

57. (New) The method of claim 44 wherein the organotypical gene encodes an organotypical marker.

58. (New) The method of claim 44 wherein the first nucleic acid is RNA.

59. (New) The method of claim 58 wherein the RNA comprises mRNA.

APPENDIX: COMPLETE CLAIMS ACCORDING TO PRESENT AMENDMENT

21. (Amended) The method of claim 43 wherein the first and second cancer-specific nucleic acids are the same.

22. (Amended) The method of claim 43 wherein the first and second cancer-specific nucleic acids are different.

24. (Amended) The method of any one of claims 54-56 wherein the RNA comprises mRNA.

25. The method of claim 24 wherein the mRNA is not expressed in the non-cancer cell.

26. The method of claim 25 wherein the mRNA comprises all or a portion of a transcript of a gene selected from the group consisting of a CEA gene, a CK20 gene, a MUC1 gene, a tyrosinase gene and a MAGE3 gene.

27. (Amended) The method of any one of claims 54-56 wherein the DNA that is detected comprises genomic DNA selected from the group consisting of genomic DNA comprising a genomic mutation, genomic DNA comprising a gene that has undergone amplification, genomic DNA comprising a gene that has undergone loss of heterozygosity, genomic DNA comprising a translocated gene and genomic DNA comprising a gene polymorphism.

28. (Amended) The method of any one of claims 54-56 wherein at least one nucleic acid that is detected comprises DNA, said DNA comprising genomic DNA selected from the group consisting of (i) the second cancer-specific nucleic acid and (ii) a cancer-associated nucleic acid that is present in at least one cancer cell in the second fraction.

29. (Amended) The method of any one of claims 54-56 wherein the DNA is genomic DNA that comprises all or a portion of an oncogene.

30. (Amended) The method of any one of claims 54-56 wherein the DNA is genomic DNA that comprises all or a portion of a tumor suppressor gene.

31. The method of claim 27 wherein the genomic DNA comprises all or a portion of a gene selected from the group consisting of a p53 gene, an erb-B2 gene, a c-myc gene, a K-ras gene, an RB gene, an APC gene and a DCC gene.

32. (Amended) The method of any one of claims 41-43 wherein at least one nucleic acid selected from the group consisting of a (i) first cancer-associated nucleic acid and (ii) a second cancer-associated nucleic acid comprises a coding portion of a gene selected from the group consisting of a tissue-specific gene, a metastasis-associated gene, a steroid hormone receptor gene, a drug resistance gene, an immunomodulation gene, a cell proliferation gene and an apoptosis gene, or a complementary nucleic acid thereto.

33. The method of claim 32 wherein the metastasis-associated gene encodes a gene product selected from the group consisting of an angiogenesis factor, a motility factor, a growth factor, a matrix degradation factor and an adhesion factor.

34. The method of claim 33 wherein the matrix degradation factor is selected from the group consisting of a proteinase and a proteinase inhibitor.

35. The method of claim 33 wherein the adhesion factor is an adherin.

36. The method of claim 24 wherein the mRNA encodes a gene product selected from the group consisting of bFGF, bFGF-R, VEGF, VEGF-R1, VEGF-R2, MMP2 and TIMP3.

37. (Amended) The method of any one of claims 41-43 wherein the cancer cell is removed from the body fluid by a method selected from the group consisting of microfiltration, density gradient centrifugation and antigen-specific immunoadsorption.

41. (New) A method for determining an increased risk for or presence of a disseminated cancer cell or a micrometastasizing cancer cell in a body fluid from a subject, comprising:

(a) dividing a plurality of cells into at least a first fraction and a second fraction, wherein each of the fractions comprises at least one cell and wherein the plurality of cells is from a body fluid of a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell, and wherein the second fraction comprises at least one cell that has been removed from said body fluid according to a method for isolating cancer cells from non-cancer cells;

(b) detecting in the first fraction an absence or presence of at least one first nucleic acid selected from the group consisting of a first cancer-specific nucleic acid and a first cancer-associated nucleic acid; and

(c) detecting, in the second fraction, an absence or presence of at least one second nucleic acid selected from the group consisting of a second cancer-specific nucleic acid and a second cancer-associated nucleic acid, wherein said first and second cancer-specific nucleic acids are different, wherein said first and second cancer-associated nucleic acids are different, wherein the presence of said first nucleic acid in the first fraction and an increased or decreased presence of the second nucleic acid in said second fraction relative to the presence or absence of said second nucleic acid in a non-cancer cell from the subject indicate an increased risk for having a disseminated cancer cell or a micrometastasized cancer cell.

42. (New) A method for determining an increased risk for or presence of a disseminated cancer cell or a micrometastasizing cancer cell in a body fluid from a subject, comprising:

(a) dividing a plurality of cells into at least a first fraction and a second fraction, wherein each of the fractions comprises at least one cell and wherein the plurality of cells is from a body fluid of a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell, and wherein the second fraction comprises at least one cell that has been removed from said body fluid according to a method for isolating cancer cells from non-cancer cells;

(b) detecting in the first fraction an absence or presence of at least one first cancer-specific nucleic acid; and

(c) detecting in the second fraction an absence or presence of at least one second cancer-specific nucleic acid, wherein said first and second cancer-specific nucleic acids are different, wherein the presence of said first cancer-specific nucleic acid in said first fraction and an increased or decreased presence of said second cancer-specific nucleic acid in said second fraction relative to the presence or absence of said second cancer-specific nucleic acid in a non-cancer cell from the subject indicate an increased risk for having a disseminated cancer cell or a micrometastasized cancer cell.

43. (New) A method for determining an increased risk for or presence of a disseminated cancer cell or a micrometastasizing cancer cell in a body fluid from a subject, comprising:

(a) dividing a plurality of cells into at least a first fraction and a second fraction, wherein each of the fractions comprises at least one cell and wherein the plurality of cells is from a body fluid of a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell, and wherein the second fraction comprises at least one cell that has been removed from said body fluid according to a method for isolating cancer cells from non-cancer cells;

(b) detecting in the first fraction an absence or presence of at least one first cancer-specific nucleic acid;

(c) detecting in the second fraction an absence or presence of at least one second cancer-specific nucleic acid; and

(d) detecting an absence or presence of at least one cancer-associated nucleic acid in at least one sample selected from the group consisting of (i) the first fraction and (ii) the second fraction, wherein the presence of said first cancer-specific nucleic acid and of said cancer-associated nucleic acid in said first fraction and an increased or decreased presence of said second cancer-specific nucleic acid and of said second cancer-associated nucleic acid in said second fraction relative to the presence or absence of said second cancer-specific nucleic acid and of said second cancer-associated nucleic acid in a non-cancer cell from the subject indicate an increased risk for having a disseminated cancer cell or a micrometastasized cancer cell.

44. (New) The method of any one of claims 41-43 wherein a nucleic acid selected from the group consisting of (i) a first cancer-specific nucleic acid and (ii) a first cancer-associated nucleic acid comprises an organotypical gene, and wherein the presence of at least one of said first nucleic acids comprising an organotypical gene indicates the type of malignant disease from which the cancer cell is derived.

45. (New) The method of any one of claims 41-43 wherein a nucleic acid selected from the group consisting of (i) a first cancer-associated nucleic acid and (ii) a second cancer-associated nucleic acid comprises a metastasis-associated gene, and wherein the presence of said first cancer-associated nucleic acid comprising the metastasis-associated gene indicates an increased risk that a disseminated cancer cell has the ability to metastasize, and wherein an increased or decreased presence of said second cancer-associated nucleic acid comprising the metastasis-associated gene in said cancer cell relative to the presence or absence of said second cancer-associated nucleic acid comprising the metastasis-associated gene in a non-cancer cell from the subject indicates an increased risk that a disseminated cancer cell has the ability to metastasize.

46. (New) The method of claim 45 wherein the metastasis-associated gene encodes a gene product selected from the group consisting of an angiogenesis factor, a motility factor, a growth factor, a matrix degradation factor and an adhesion factor.

47. (New) The method of claim 46 wherein the matrix degradation factor is selected from the group consisting of a proteinase and a proteinase inhibitor.

48. (New) The method of claim 46 wherein the adhesion factor is an adherin.

49. (New) The method of claim 45 wherein the nucleic acid is selected from the group consisting of DNA and RNA.

50. (New) The method of claim 49 wherein the RNA comprises mRNA.

51. (New) The method of claim 50 wherein the mRNA encodes a gene product selected from the group consisting of bFGF, bFGF-R, VEGF, VEGF-R1, VEGF-R2, MMP2 and TIMP3.

52. (New) The method according to any one of claims 41-42 wherein steps (a) - (c) are performed before and after administering a candidate anticancer therapy to a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell.

53. (New) The method according to claim 43 wherein steps (a) - (d) are performed before and after administering a candidate anticancer therapy to a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell.

54. (New) The method of claim 41 wherein the first nucleic acid is RNA and wherein the second nucleic acid is selected from the group consisting of DNA and RNA.

55. (New) The method of claim 42 wherein the first cancer-specific nucleic acid is RNA and wherein the second cancer-specific nucleic acid is selected from the group consisting of DNA and RNA.

56. (New) The method of claim 43 wherein the first cancer-specific nucleic acid is selected from the group consisting of DNA and RNA, wherein the second cancer-specific nucleic acid is selected from the group consisting of DNA and RNA, and wherein the cancer-associated nucleic acid is selected from the group consisting of DNA and RNA.

57. (New) The method of claim 44 wherein the organotypical gene encodes an organotypical marker.

58. (New) The method of claim 44 wherein the first nucleic acid is RNA.

59. (New) The method of claim 58 wherein the RNA comprises mRNA.

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